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Cytoarchitectonic and quantitative Golgi study of the hedgehog supraoptic nucleus

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ABSTRACT

A cytoarchitectural study was made of the supraoptic nucleus (SON) of the hedgehog with special attention to the quantitative comparison of its main neuronal types. The main purposes were (1) to relate the characteristics of this nucleus in the hedgehog (a primitive mammalian insectivorous brain) with those in the SONs of more evolutionarily advanced species; (2) to identify quantitatively the dendritic fields of the main neuronal types in the hedgehog SON and to study their synaptic connectivity. From a descriptive standpoint, 3 neuronal types were found with respect to the number of dendritic stems arising from the neuronal soma: bipolar neurons (48%), multipolar neurons (45.5%) and monopolar neurons (6.5%). Within the multipolar type 2 subtypes could be distinguished, taking into account the number of dendritic spines: (a) with few spines (93%) and (b) very spiny (7%). These results indicate that the hedgehog SON is similar to that in other species except for the very spiny neurons, the significance of which is discussed. In order to characterise the main types more satisfactorily (bipolar and multipolars with few spines) we undertook a quantitative Golgi study of their dendritic fields. Although the patterns of the dendritic field are similar in both neuronal types, the differences in the location of their connectivity can reflect functional changes and alterations in relation to the synaptic afferences.

INTRODUCTION

The underlying connectivity of the neurosecretory process has been one of the main objectives in research on the hypothalamic neurosecretory nuclei. For this reason, most studies on the supraoptic nucleus (SON) have been performed using light microscope cytoarchitectonic techniques (Enestron, 1967; Christ, 1968; Machín, 1975). Neurosecretion is one of the most important functional roles of the SON. The vasopressin and oxytocin hormones which are synthesised in the SON, are carried through the axons of the SON neurons (supraoptic-hypophysial tract) to the neurohypophysis (Legait et al. 1966; Swaab et al. 1975; Vandesande & Dierickx, 1975).

Descriptions of supraoptic neuronal types, using the Golgi techniques, have been scanty due to the difficulty of staining these cerebral centres. However, studies in the mouse (Krieg, 1932), monkey (Fox & Zabors, 1960; Luqui & Fox, 1976), cat, guinea pig (Le Franc, 1966), dog (Leontovich, 1970), rat (Dyball et al. 1979; Dyball & Kemplay, 1982; Bruni & Perumal, 1984), and rabbit (Felten & Cashner, 1979), have established the general morphological characteristics of the supraoptic neurons. The species that have been studied belong to different evolutionary mammalian lines, phylogenetically distant to a greater or lesser extent, to primitive mammals such as the insectivora. We therefore considered that a study of the neuronal characteristics of the SON in the hedgehog (*Erinaceus europaeus*) would be of interest when evaluating both the phylogenetic variability of this nucleus and the possible functional specialisations inferred from the morphological characteristics of the neuronal types. Furthermore, although Golgi material has been widely used for quantitative studies on connectivity in other cerebral areas, such studies are less numerous for hypothalamic neuronal populations. The Golgi method gives a good characterisation of the neuronal types in the SON, especially with regard to the neuronal dendritic field. Since the dendritic field is determined both by intrinsic or genetic and extrinsic

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factors, the latter related to the surrounding connective field, a quantitative study of dendritic trees should give further information about both factors for specific neuronal types. Accordingly, we made a descriptive study of the main neuronal types in the hedgehog SON and compared these with those of other species. In addition, we carried out a quantitative study of the dendritic field of the main SON neuronal types in the hedgehog, in order to characterise them better as well as to obtain further information about their phylogenetic significance and their functional role in synaptic connectivity within this nucleus.

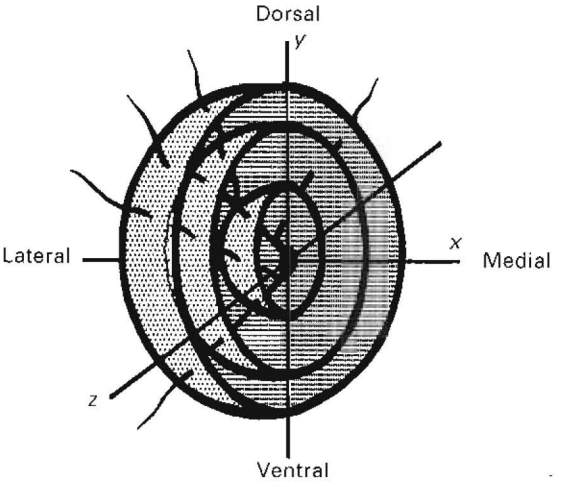

Fig. 1. Schematic representation of the method used to calculate the dendritic density around the soma. Dendritic density is obtained by the intersections between the dendritic branches and imaginary spheres created by the computer. The centre of the spheres is the neuronal soma. The radius was increased by a constant value (20 μm).

MATERIAL AND METHODS

Eight male adult hedgehogs (600–700 g) were used. All animals were anaesthetised with an intraperitoneal injection of pentobarbital sodium and perfused transcardially with isotonic saline solution followed by 10% formaldehyde (neutralised pH 7.2). The brain was removed and the hypothalamus was stained using general topographic and cytological methods (cresyl violet and Klüver-Barrera) in 3 animals, and by the rapid Golgi procedure (Cajal & de Castro, 1972) in the other 5. Transverse sections were cut at 8 μm for the specimens from the first 3 brains and at 200 μm in the 5 Golgi-stained brains. The localisation of the supraoptic neurons was undertaken according to previous cytoarchitectonic studies on the hedgehog hypothalamus by Gil & Machín (1983).

Two types of study were made on a selection of 170 Golgi stained neurons: (1) an initial descriptive study of their general characteristics and (2) a quantitative study establishing 2 aspects in the dendritic field: (a) the dendritic spine number, expressed as spines/mm, and (b) the topological and metric parameters of the dendritic field. Measurement of the topological and metric parameters of the dendritic field was performed using a computer system (Abella et al. 1985). Input to this sytem treats the neuron 3-dimensionally as a dichotomous tree. Prior to the computerised analysis the following procedures were used: (1) the neurons were drawn using a camera lucida coupled to an optical microscope; (2) the relevant points of neuronal morphology were selected (i.e. soma, inflection and intermediate points, bifurcation and terminal points); (3) the 2-dimensional coordinates (x,y) of each of these points were obtained by means of a graphics board connected to the computer system; (4) the 3rd coordinate (z) was obtained by means of a sensor which measure the vertical displacement of the microscope stage (Militron, Feinpüf). The sensor was connected to the computer system.

The basic functions of the computer system are: (1) centrifugal dendrite ordering; (2) neuron analysis in order to obtain parameters. The latter included (*a*) dendritic length; (*b*) total number of dendritic branches per stem and number of branches of different orders per neuron; (*c*) dendritic density of the neuron and dendritic trees based on Sholl's analysis (Sholl, 1953) – this method evaluates the cellular dendritic field by counting the dendritic intersection points through imaginary spheres drawn at regularly increasing radii from the neuronal body, in this study with the radii increasing by 20 μm; (*d*) spatial distribution of dendritic density, in this study by counting dendritic intersection points with Sholl's spheres in 4 spherical sectors: dorsal, ventral, lateral and medial, all starting from the neuronal soma (Fig. 1). (3) Statistical analysis of the results using Student's t test for comparison of median values for dendritic spines and branches, the χ^2 test to compare frequencies of branches of different orders and dendritic density distributions, and analysis of variance for 2 factors: soma distance (repeated measures) and neuronal type (bipolar-multipolar neurons). The Scheffé test for a post hoc comparison of dendritic density was performed.

RESULTS

The SON is a paired nucleus located rostrally in the anterior hypothalamus in a ventrolateral position above the optic chiasm (Fig. 2). This nucleus is

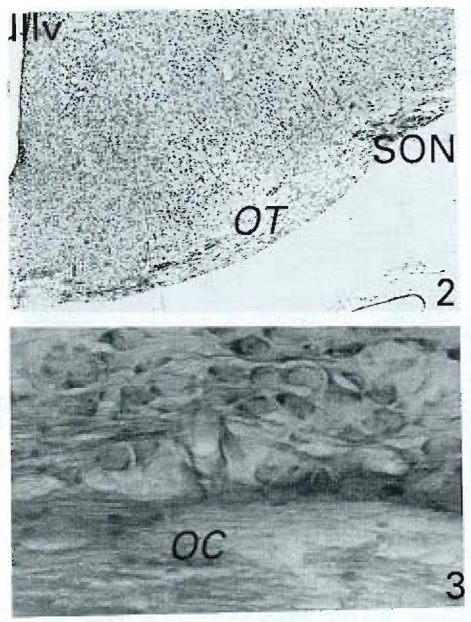

Fig. 2. A survey view of the supraoptic region at caudal levels, where the supraoptic nucleus (SON) can be observed above and lateral to the optic tract (*OT*). IIIv: 3rd ventricle. Nissl stain. × 70.

Fig. 3. Detail of the neurons of the supraoptic nucleus and their close relation to the optic chiasm (*OC*). Klüver-Barrera. × 700.

elongated along a ventrolateral axis and has a comparatively higher neuronal density in its most dorsolateral area.

The SON measures 958 μm in its anteroposterior extent. The greatest volume is present in its rostral part, gradually decreasing caudally. The nucleus has very precise limits and is directly related to the optic tract in the middle lateral area. Fibres can be observed coming from the optic tract between the neurons by light microscopy (Fig. 3). SON neurons stained with cresyl violet have an average diameter of 15–20 μm with a fusiform or rounded shape and show abundant peripherally distributed accumulations of chromatin. The nucleus of these neurons is mainly rounded with an average diameter of 10 ± 2 μm, is generally situated laterally in the cytoplasm and has an obvious nucleolus.

Descriptive Golgi study

The SON neurons have been classified according to the number of dendritic trees that arise from the soma. We found 3 neuronal types: (1) bipolar neurons with 2 dendrite stems; (2) multipolar neurons with more than 2 dendritic stems; and (3) monopolar neurons, with only a single dendritic stem. The 2 most common neuronal types were bipolar (48%) and multipolar (45.5%). Since monopolar neurons appeared only occasionally, they were not considered in our quantitative study.

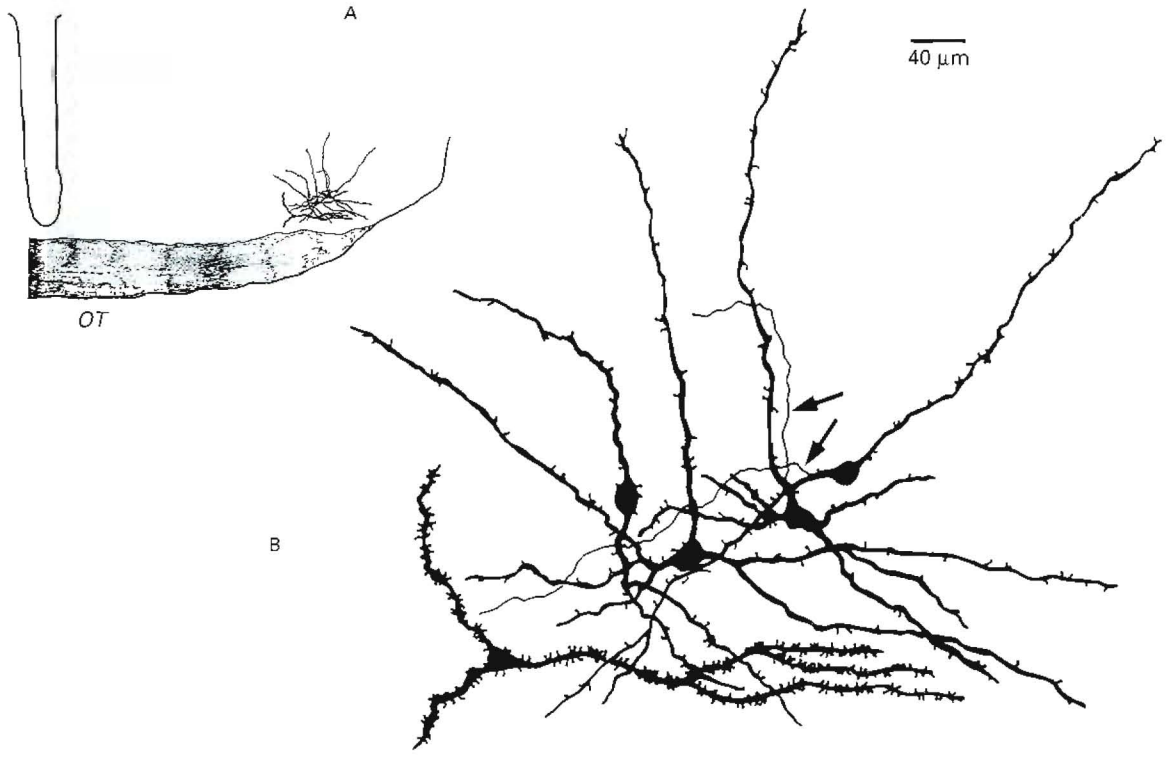

Fig. 4. Camera lucida drawing of the bipolar and multipolar neuron groups of the hedgehog supraoptic nucleus; *OT*, optic tract. A, location within the nucleus. B, high magnification drawing that depicts the main morphological characteristics of the neuronal types studied. Arrows, axons.

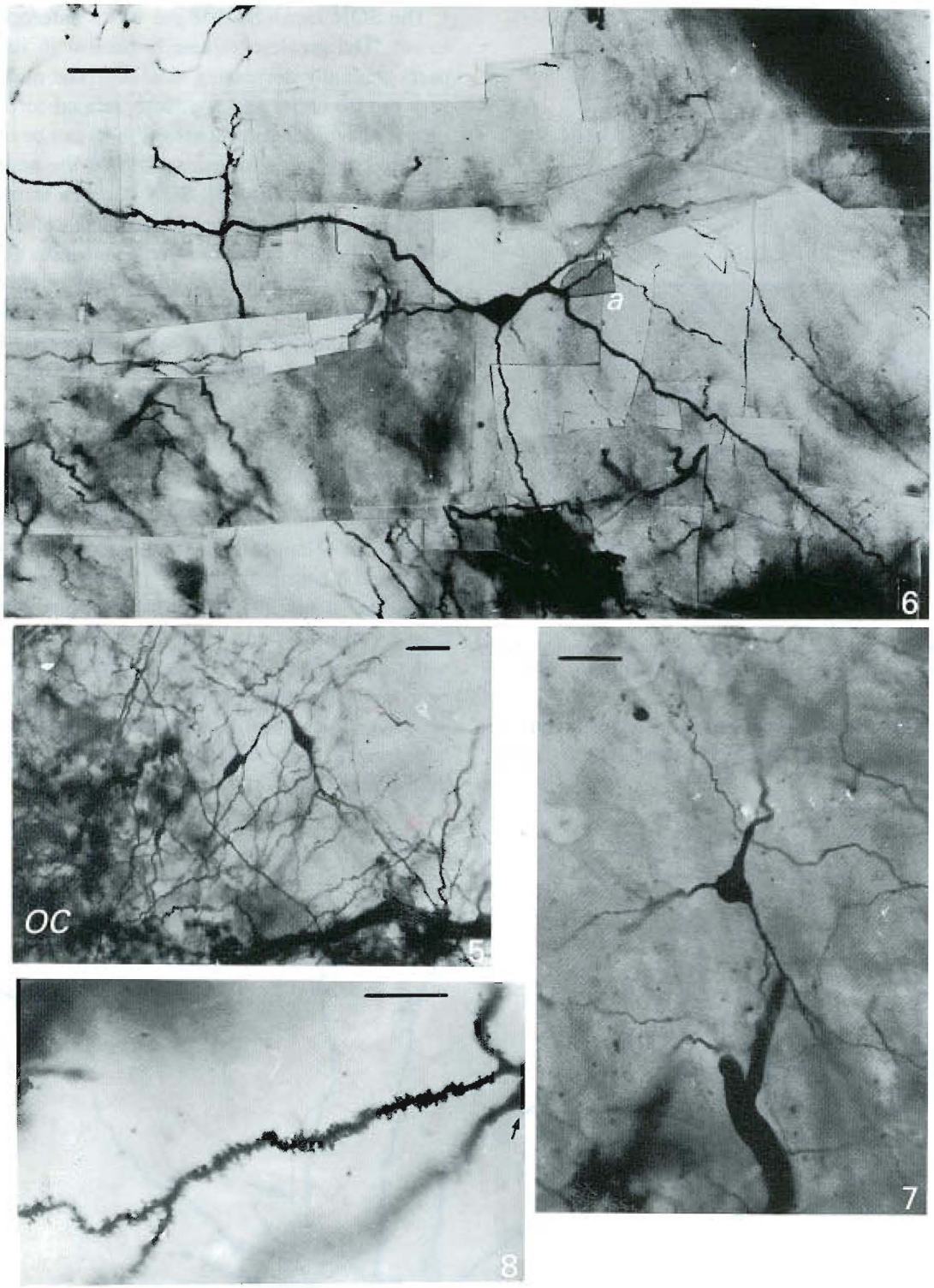

Fig. 5. Bipolar neurons of the supraoptic nucleus in close proximity to the optic chiasm (*OC*). Golgi stain. Bar, 50 μm.

Fig. 6. Photomontage of a multipolar neuron of the supraoptic nucleus with a fusiform soma from which 3 dendritic stems originate. Golgi stain. *a*, axon. Bar, 50 μm.

Fig. 7. Multipolar neuron of the supraoptic nucleus. Golgi stain. Bar, 50 μm.

Fig. 8. Detail of a dendritic stem belonging to a very spiny multipolar neuron. Arrow, neuronal soma. Golgi stain. Bar, 50 μm.

Bipolar neurons are characterised by a fusiform or round neuronal body (Figs 4, 5) with a variable number of somatic spines. The 2 dendritic trees start at opposite ends of the soma and are normally straight, although they sometimes twist and turn. There is little dendritic branching, and there are only

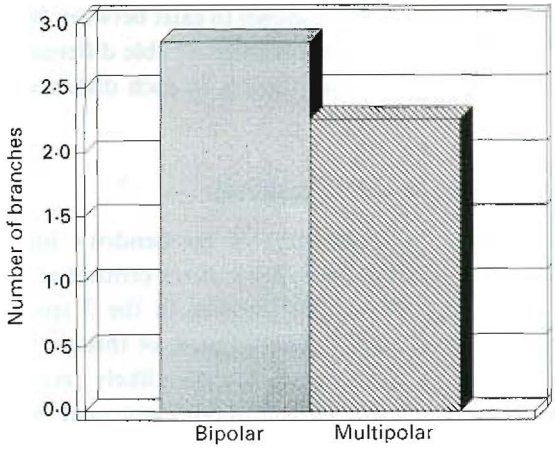

Fig. 9. Mean number of dendritic branches per stem in bipolar and multipolar neurons.

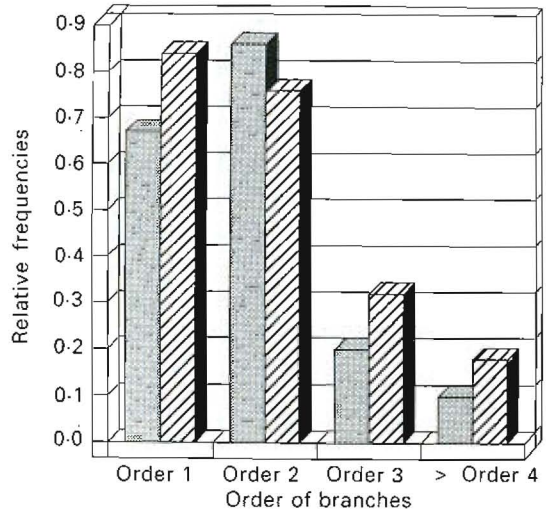

Fig. 10. Relative frequencies of branching orders in bipolar (matt columns) and multipolar neurons (cross hatched columns).

a few dendritic spines, suggesting a poor dendritic field. These dendritic spines are principally mushroom-shaped and are obliquely oriented in relation to the dendrite. Axons arise either from the soma or proximal dendritic regions and are mostly oriented towards the supraoptic-hypophysial tract.

Two types of multipolar neurons were identified according to the number of dendritic spines. The most frequent (93%) shows few spines (like those found in the bipolar neurons) and has an irregular, polygonal fusiform or rounded soma (Figs 4, 6, 7). The somatic spines are scarcer in multipolar than in bipolar neurons. The latter normally shows 3 dendritic stems with poor ramification, although some neurons do reach 6th order branching. Axons arise from either the neuronal body or the neighbouring dendrite branches of 1st and 2nd order and are directed towards the supraoptic-hypophysial tract or sometimes to a lateral area. It is to be noted that only a small part of the axon could be visualised. This type of neuron has been analysed in this quantitative study because of its large numbers within the neuronal population.

A second multipolar type was found ventrally within the nucleus (Fig. 4). These neurons are characterised by a high spine density in the soma as well as in the dendritic stems (Fig. 8). They have a fusiform or triangular neuronal soma with 3 dendritic stems unequally branched and occasionally reaching 4th order. The orientation of the neurons with respect to the SON is mainly horizontal and they principally occupy medial or lateral regions. It was difficult to see axons for this type of neurons. The scarcity of their number did not permit a statistical study.

Quantitative study of the dendritic field of the main neuronal types

Number of dendrite spines

A total of 117 dendritic stems longer than 80 μm were selected and the number of dendritic spines were counted. The data obtained were then divided by the dendrite stem length in order to obtain the quotient spine number/mm. The average found in the bipolar neurons was 45 ± 3.4 spines/mm, while the average in the multipolar neurons was 43 ± 2.7 spines/mm, illustrating the low number of receptive structures in these neuronal types. Statistical comparison by Student's t test showed no significant difference.

Dendritic field branching

Number of dendritic branches by stem. Figure 9 shows that the bipolar neurons have 2.85 ± 0.26 branches per dendritic stem, while the multipolar ones have 2.26 ± 0.22 in a total of 40 and 65 studied stems, respectively. Statistical comparison of both values by Student's t test showed no significant differences.

Number of branches of the different orders. Figure 10 shows the relative frequency of number of branches per order in both bipolar and multipolar neurons. Branches of order one and two are the most common in both types of neuron, whereas other orders are less numerous. Comparing the absolute value of both neuronal types by means of the χ^2 test, no statistically significant differences were found.

These results show that the degree of branching is basically similar in both neuronal types.

Dendritic density around the neuronal body

The dendritic density has been quantified at 20 μm intervals from the soma until 240 μm radii are reached.

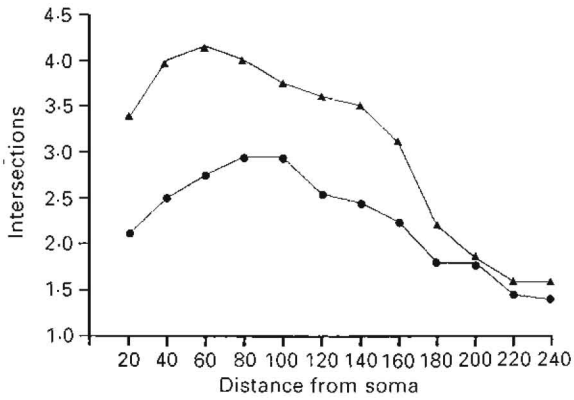

Fig. 11. Distribution of dendritic density per neuron around the soma in bipolar (circles) and multipolar (diamonds) neurons.

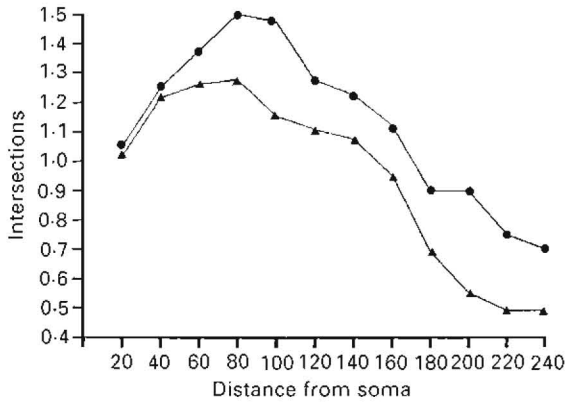

Fig. 12. Distribution of dendritic density per dendritic stem around the soma in bipolar (circles) and multipolar (diamonds) neurons.

Sholl's spheres, as described in Material and Methods, were used for this study.

Greater dendritic density values were obtained for multipolar than for bipolar neurons. Analysis of variance indicated the existence of statistically significant differences between the dendritic field densities of bipolar and multipolar neurons ($P < 0.001$). This analysis also indicated the existence of a statistically significant neuronal type by distance from the soma interaction ($P < 0.0001$). As can be seen in Figure 11, multipolar neurons showed greater dendritic density around the neuronal soma than bipolar neurons. The subsequent Scheffé analysis revealed statistically significant differences between soma and 160 μm from it ($P < 0.01$). These results were expected because of the greater dendritic stem number of the multipolar neurons.

With the purpose of obtaining a more precise comparison between both neuronal types the distribution of dendritic density around the soma was studied tree by tree. The results are shown in Figure 12 where a great similarity is shown to exist between both neuronal types with no statistically reliable differences present in the values with respect to each distance.

Orientation of the dendritic density

By following a quantification of the dendritic intersections with Sholl's concentric spheres centred at the soma, dendritic density distribution in the 3 spatial dimensions was studied. The purpose of this part of the investigation was to know the most likely areas of branching in the dendritic field of the 2 neuronal types studied.

As shown in Figure 13, the distribution of dendritic density in the 4 sections considered is much more homogeneous in the multipolar than in the bipolar neurons. The latter have more density in the lateral and medial areas, indicating that their orientation is predominantly horizontal.

Statistical comparison of the absolute value of the dendritic intersection points with Sholl's spheres by means of the χ^2 test shows significant differences in these orientations ($P < 0.001$). The ratio of dendritic density of the bipolar neurons is significantly greater in the medial area and smaller in the ventral area. This different distribution of the dendrites into SON can implicate differences in the connectivity of these 2 neuronal types.

DISCUSSION

Both bipolar and multipolar neurons are the main neuronal types in the hedgehog supraoptic nucleus. These data concur with several previous Golgi studies in cat (Le Franc, 1966), monkey (Luqui & Fox, 1976), rabbit (Felten & Cashner, 1979) and rat (Dyball et al. 1979; Bruni & Perumal, 1984) which give neuronal descriptions similar to ours. However, they differ from those of Krieg (1932), which showed only multipolar neurons in the rat SON, and those of both Leontovich (1970) and Dyball & Kemplay (1982), which showed mostly bipolar neurons in the rat and newborn dog. These differences may reflect the phylogenetic stage of the species, but in spite of the primitivism of the Insectivora within the placental mammals (Romer, 1974), the neuronal types in the hedgehog SON are the same as those found in other phylogenetically more advanced species, suggesting evolutionary stability in this nucleus.

An important aspect for discussion is the meaning of the very spiny multipolar neurons which are very infrequent in the SON of the hedgehog. The character-

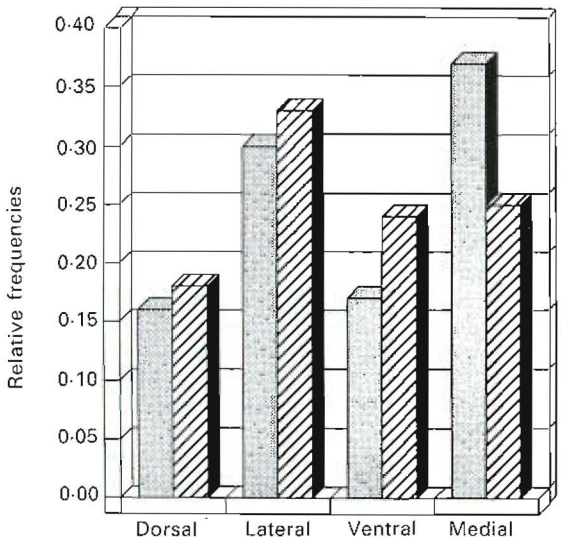

Fig. 13. Distribution of dendritic density within 4 sectors of the supraoptic dendritic field for bipolar (matt columns) and multipolar (cross hatched columns) neurons.

istics of their dendritic fields indicate that these neurons have a greater number of afferences than other neuronal types, and that this reception occurs in special areas of their oriented dendritic field. Moreover, the presence of very spiny multipolar neurons could indicate that the complexity of the connectivity in the hedgehog SON may be greater than that described for other species.

The quantitative description has given a better determination of the dendritic field of the studied neurons. Unfortunately, it is not possible to compare our data with similar studies because none exist. Our results can therefore only be related to previous qualitative data in a general way. Dendrite spine number is expressed by the quotient spine number/mm. Both neuronal types show similar low values particularly when they are compared with neuronal types that are clearly spiny. Even though some studies have indicated that there is a relative scarcity of spines in these supraoptic neurons (Leontovich, 1970; Luqui & Fox, 1976), none of them give numerical values. The variation in the number of dendritic spines with the age of animals and physiological conditions raises the question of the homogeneity in our samples. Our mean values of the number of spines have been very similar between and within animals. This result differs from that reported by Bruni & Perumal (1984) which shows a very high spine variability in rat SON neurons.

With regard to the degree of dendrite branching, or results show a sparse branching pattern and conform with previous studies on the SON. Nevertheless, our number of branch orders exceeds that in the studies of Leontovich (1970) and Felten & Cashner (1979), which indicate a maximum branching order of 3. The comparison between the dendritic branching patterns in bipolar and multipolar neurons reveals no quantitative differences and shows that they have a basically similar outline. In the multipolar neurons dendritic density is higher than in bipolar neurons because of their greater number of dendritic stems; however, the pattern of the dendritic field is similar in each dendritic stem.

Considering the characteristics of this dendritic field from a phylogenetic standpoint, the main neurons studied in this paper fall within the most primitive types within the mammalian central nervous system and represent the generalised or reticular (Leontovich & Zhukova, 1963) or the isodendritic types (Ramón Moliner, 1962, 1966; Ramón Moliner & Nauta, 1966). The fact that the descriptive and quantitative characteristics are similar in bipolar and multipolar neurons, when analysed tree by tree, may indicate a clear evolutionary relationship between both neuronal types. Conversely the very spiny multipolar neurons do not clearly fulfil the conditions of the isodendritic type because of their highly receptive field, which suggests a greater degree of evolution and specialisation.

The distribution study of the dendritic density around the soma has made it possible to plot the orientation of the dendritic field in bipolar and multipolar neurons. This orientation is related to the connectivity of the neurons in the nucleus. Statistical differences in dendritic distribution suggest that the neuronal dendrites are directed towards specific areas in the nucleus in such a way that bipolar neurons make contact principally with the lateral and medial areas (horizontal orientation). Multipolar neurons make their contacts in a more radial fashion. We must emphasise the great contrast between some results of previous studies and our own. Leontovich (1970), for instance, indicated a principal dorsoventral orientation in connectivity of SON bipolar neurons in the dog. McNeill & Sladek (1980) and Armstrong et al. (1982) found an important dendrite content in a dorsoventral direction in the rat SON.

Considering that there is a correlation between the number of afferences and the density of a dendritic field, it can be inferred that bipolar neurons have a mainly horizontal connectivity, whereas multipolar neurons have a mainly radial connectivity. Therefore, although the patterns of the dendritic fields are similar, their connectivity differs, being more specific than in multipolar than in bipolar neurons. It could the considered that they are 2 very similar neuronal types or perhaps constitute only one neuronal type

that is specialised towards different connectivities throughout the evolutionary process. It is important to note the difficulty in correlating the preferential orientation areas of dendritic density to specific kinds of afferences since they are sometimes very specific (Cunningham & Sawchenko, 1988). Besides, in view of earlier papers, it is very difficult to determine the real magnitude of the intrinsic connective fields with respect to the extrinsic ones in the SON (Koizumi & Yamashita, 1972; Leng, 1982; Lerant et al. 1975; Nicoll & Baker, 1971; Zaborszky et al. 1975; Ray and Choudhury 1990).

Since the anatomical results do not always correspond to the electrophysiological ones, only those connectivity areas that are well defined by previous studies are indicated. Hayward (1977), Leng (1982) and Hatton et al. (1983) reported the existence of a high connectivity field in the dorsal and perinuclear area of this nucleus. Anderson et al. (1990) also found a great number of dorsal afferences to the nucleus with exclusive connections to this area from different centres. Among the extrinsic afferences to the SON the largest contingent is the medial forebrain bundle (Lerant et al. 1972; Zaborszky et al. 1975). This bundle, found dorsally to the SON, receives rising afferences from the cerebral trunk as well as others descending from the septum and olfactory tubercles. Thus it is possible to determine a principal dorsal connectivity which has been found in this study (Anderson et al. 1990).

The hippocampus fornix has a similar position and provides 13% of the extrinsic SON afferences in the rat (Zaborszky et al. 1975). The large contingent of dorsal connectivity found in supraoptic neurons with respect to the ventral area may be explained by all these bundles.

In the rat SON ventral surface, McNeill & Sladek (1980) and Armstrong et al. (1982) found, by means of immunocytochemical methods, high catecholaminergic afferences that could be important in the regulation of the neurosecretory function of the neurons in this nucleus. However, we did not find an especially increased dendritic density towards the ventral fields in the studied neurons.

A last point to be mentioned, taking into account the fact that the studied neurons in the SON are closely related, is their similar plasticity under certain conditions. With respect to this fact, Dyball and Graten (1988) found similar changes in bipolar and multipolar dendritic fields under increasing afferences. In the same manner, changes in the afferent input to the hedgehog SON produce a significant and similar increase in both bipolar and multipolar dendritic fields (Sanchez-Toscano et al. 1989). In spite of the different orientation of their receptive field, the behaviour of the dendrites is quite similar. Moreover, this fact may indicate a relationship among the intrinsic functional properties of these neurons.

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